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Selection for increased anther culture response in maize

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Summary. Anther culture of a three-way cross, (H99 × FR16) × Pa91, resulted in the regeneration of two anther-derived plants which were crossed to produce an F_1 progeny. Fourteen S_1 families derived from this cross were evaluated for their anther culturability. Dramatic increases in the level of androgenesis, expressed as the percentage of cultured anthers which produced embryo-like structures, were observed. An overall mean response frequency of 23.4% was observed for the S_1 families. This was compared to a 3.5% response in the original three-way cross. These results demonstrate that genetic improvement of in vitro androgenesis in maize is possible and that anther culture per se constitutes a procedure for selecting genes which favor increased levels of response.

Key words: Androgenesis – Haploids – Plant tissue culture

Introduction

Reports of the occurrence of haploids in maize date back to the 1930's (Stadler 1931; Randolph 1938). Since that time there has been much discussion about the potential utilization of haploids in maize breeding (Brown 1953; Sprague 1967; Chase 1969). Since maize breeding is concerned with the development of inbred lines to use as parents of productive hybrids, the rapid advance to homozygosity which accompanies the doubling of haploids is an attractive feature (Chase 1972). However, attempts at utilizing haploids in maize breeding have been frustrated by the lack of a reliable means of generating sufficient numbers of doubled haploid lines from a broad spectrum of commercially-useful germplasm.

Anther culture represents a potentially powerful method whereby large numbers of haploid individuals can be produced directly from microspores in vitro (Wu 1986; Keller et al. 1987). Unfortunately, response frequencies in cultured maize anthers have been very low in all but a few genotypes (Ku et al. 1978; Genovesi and Collins 1982; Dieu and Beckert 1986; Petolino and Jones 1986). The utilization of microspore-derived lines in commercial maize breeding will require increased responsiveness to anther culture in agronomically acceptable germplasm.

The highly significant genotypic effects observed in some studies (Ku et al. 1978; Genovesi and Collins 1982; Dieu and Beckert 1986; Petolino and Jones 1986) suggest that genetic factors are important in determining the level of response to anther culture in maize. Increasing the anther culturability of commercially-important germplasm via genetic means has been suggested (Petolino and Thompson 1987). Recent evidence indicates that parents which give rise to highly responsive hybrids can be identified and genetic improvement is possible through selection (Petolino and Thompson 1987).

The anther culture process itself may represent an effective selection criterion for genes favoring in vitro androgenesis. The recombination of microspore-derived individuals may result in genotypes exhibiting enhanced response to anther culture. This paper reports on the selection of maize germplasm with high levels of anther culturability resulting from inter-mating microspore-derived lines.

Materials and methods

The inbred lines used as parents in this study were H99, FR16, and Pa91. Seed was obtained from Holden's Foundation Seeds, Williamsburgh, USA (H99 and Pa91) and Illinois Foundation

Seeds, Tolono, USA (FR16). All lines were maintained by self-pollination for two years prior to being used for crossing. The three-way cross, (H99 × FR16) × Pa91, was made by controlled pollination.

Using previously published procedures (Petolino and Thompson 1987), anthers from representative tassels of (H99 × FR16) × Pa91 were cultured during the summer of 1985. Two anther culture-derived embryos, obtained from two separate tassels, regenerated plants which were grown to maturity in the greenhouse during the winter of 1985. One plant (#139) produced an ear shoot and a tassel with no anther extrusion. A second plant (#39) produced viable pollen but the ear shoot was late in its development. The pollen from plant #39 was applied to the silks of plant #139 resulting in the formation of a single F_1 hybrid (139/39) seed. The seed was germinated and the resulting plant was self-pollinated to produce an F_2 population. The F_2 population was grown in the field during the summer of 1986 and 14 random self-pollinations were made and grown ear-to-row. The resulting 14 S_1 families were evaluated for their anther culturability during the summer of 1987.

All plants used for anther culture were field grown during July to August in Champaign, USA. Tassels with anthers containing late uninucleate-early binucleate microspores, as determined by mithramycin/fluorescent staining (Pace et al. 1987), were removed from donor plants prior to emergence from the whorl. Tassels were then processed and anthers cultured as previously described (Petolino and Thompson 1987). A total of 16,140 and 12,600 anthers from 53 and 70 individual plants were cultured from the three-way cross and the S_1 families, respectively. Response frequency was expressed as the percentage of cultured anthers that produced at least one embryo-like structure after six weeks in culture.

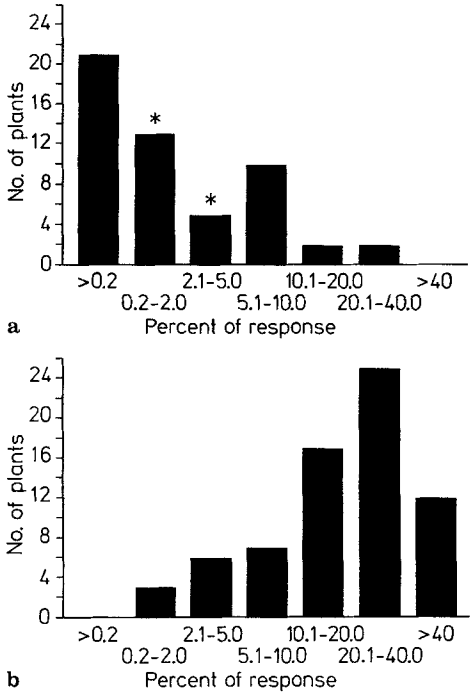

Fig. 1 a and b. Frequency distribution of anther culture response from individual maize plants of **a** (H99 × FR16) × Pa91 (n = 53), and **b** 14 S_1 families (n = 70); * donor plants from which #39 and #139 were regenerated

Results and discussion

A distribution of response frequencies from individual plants from the three-way cross, (H99 × FR16) × Pa91, is presented in Fig. 1 a. The response frequencies are skewed toward the lower values and the overall mean response is 3.5%. Only 4 of the 53 (7.5%) plants displayed anther culture response frequencies greater than 10%. The mean frequency is somewhat lower than observed in earlier work with this cross (Petolino and Jones 1986) but the range of response is consistent with previous findings (Petolino and Thompson 1987).

Individual anther culture response frequencies of the S_1 plants are presented in Fig. 1 b. A dramatic shift toward increased anther response was observed. The overall mean response frequency for the S_1 plants was 23.4%. A total of 54 of the 70 (77.1%) S_1 plants evaluated had response frequencies greater than 10%. Means for the 14 S_1 families ranged from 9.2% to 41.3% (Table 1). The level of anther culture response observed in this material represents the highest reported to date for maize.

These results demonstrate that genetic improvement of in vitro androgenesis in maize is possible and that anther culture per se constitutes a procedure for selecting genes which favor increased levels of response. A single cycle of selection resulted in greater than a six-fold increase in anther culture responsiveness as measured by the percentage of anthers producing embryo-like structures. This phenomenon was also observed by Chase (1972) who noted that maternal haploid derivatives displayed higher rates of parthenogenesis than the source stocks from which they were derived.

Selecting individuals based on their individual plant response to anther culture may not be a practical means of increasing androgenesis in subsequent generations.

Table 1. Mean anther response from cultured anthers of 14 S_1 families of maize

Genotype	Anthers plated	Response (%)
139/39-01	900	41.3
139/39-02	900	32.6
139/39-03	900	20.2
139/39-04	900	22.0
139/39-05	900	34.6
139/39-06	900	16.6
139/39-07	900	15.0
139/39-08	900	9.2
139/39-09	900	30.9
139/39-10	900	12.5
139/39-11	900	25.4
139/39-12	900	18.3
139/39-13	900	22.3
139/39-14	900	26.6

The need to sample within a genotype due to the non-genotypic, plant-to-plant variation in anther culture response (Pace et aL. 1987; Petolino and Thompson 1987) makes this approach difficult. The two original plants from which the tassels were harvested (Fig. 1 a), that ultimately lead to the regeneration of plants #39 and #139, were not among the most productive based on their individual anther response (0.8% and 4.4%, respectively). However, the inter-mating of microspore-derived plants appears to be an effective means of shifting allelic frequencies toward increased responsiveness.

The enhanced anther culturability of commercially-important germplasm represents an important step toward the utilization of haploids in maize breeding. Improvements in the efficiency of plant regeneration and chromosome doubling, through a combination of genetic and cultural approaches, would further broaden the applicability of this technique.

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